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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/824,786	04/15/2004	Akwasi Minta	IVGN 894	6657

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EXAMINER	
SODERQUIST, ARLEN	

ART UNIT	PAPER NUMBER
1797	

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01/10/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/824,786	MINTA ET AL.	
	Examiner	Art Unit	
	Arlen Soderquist	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 2* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 2* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6-18-04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 1-2 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsien (US 5,049,673) in view of Poenie (US 5,576,433). In the patent Tsien teaches fluorescent indicator dyes for calcium working at long wavelengths. The dyes, named Rhod-1, Fluo-1, Rhod-2, Fluo-2 and Fluo-3 are shown in figures 2-2 and 3-2. All of the dyes are based on the BAPTA ligand coordinating group (abstract, column 4, and column 6). Fluo-3 is substantially similar to the instant claims. The structure of Fluo-3 is within the scope of the structure shown at the top of column 7. Column 7, line 37 shows the variability contemplated for that position of the molecule. Column 10, line 48 to column 11 line teach several advantages for these dyes compared to previous dyes indo-1, quin-2 and fura-2. The visible excitation reduces the cost of measuring with the dyes compared to the UV excitation of the prior dyes. The visible excitation will result in reduced autofluorescence and for Fluo-3 the quantum efficiency X the extinction coefficient is slightly less than the value for fura-2. Column 12, lines 34-37 teaches that Fluo-3 is generally preferred of the Fluo-dyes. The primary difference between the instant structures and the Fluo-3 structure is in the X group.

In the patent Poenie teaches fluorescent calcium indicators targeted to specific intracellular environments. The patent discloses several new calcium indicator dyes which are retained in the cytosol or which bind to cellular membranes and report calcium levels near the membrane. These indicators are analogs of a previously described tetracarboxylate calcium

indicator fura-2 and indo-1 where the 5' methyl of the BAPTA moiety is replaced with a derivative of propionic acid in the form of --CH_2CH_2CON--($CH_2)_n$ --$N(X_1)$ X_2, where n is 4. The propionic acid derivative provides a flexible means for introducing additional functionality to the calcium indicator without greatly impacting the fluorescence and ion chelating properties of the parental indicator. When X.sub.1 is CH_2COOH and X_2 is part of a ring system like piperazine, a fluorescent calcium indicator, Fura-PE3, is obtained which resists compartmentalization and leakage out of the cytosol. The tertiary amino group survives subsequent acetoxymethyl esterification of the hexacarboxylate form of the indicator without quaternization. In addition, the tertiary amino group can ionize once the indicator is inside the cell giving a zwitterion. All the zwitterionic calcium indicators reported herein resist leakage and compartmentalization. When X_1 is a sufficiently hydrophobic alkyl chain, as in $C_{12}H_{25}$, and X_2 is part of a ring system like piperazine, FFP-18, the resulting indicator binds to membranes and reports calcium levels near the membranes. Each of these indicators are derived from a new BAPTA-based chelator moiety which serves as a convenient and flexible starting point for generating a wide variety of new calcium indicators while retaining the ion selectivity and pH insensitivity of BAPTA. Columns 1-2 teach that the introduction of fura-2 and indo-1 provided revolutionary new approaches for studying calcium in individual cells. These indicators were much brighter than their predecessor, quin2, and made it possible to measure calcium based on the ratio of fluorescence intensity at two excitation or emission wavelengths. The rapid adoption of fura-2 and its relatives, and the experience gained in using these indicators also quickly revealed several problems related to dye loading, such as leakage or compartmentalization, spectral alterations between intracellular dye and that in free solution and unwanted binding to cellular constituents. Furthermore, it has become apparent that fura-2 does not always faithfully report rapid changes in calcium seen in some excitable cells and may altogether miss some rapid and/or highly localized calcium transients. The one indicator which did not suffer these problems was rhod-2, which, unlike the other indicators, contained a positive charge. This suggested that a zwitterionic indicator might be less susceptible to leakage and compartmentalization. The use of rhod-2 would seem to be a good solution to the problem but

unfortunately, rhod-2 exhibits neither the spectral shift seen with fura-2 nor the large increase in fluorescence exhibited by fluo-3.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the BAPTA structure of the Fluo-3 taught by Tsien in the manner taught by Poenie because of the large increase in fluorescence as taught by both Tsien and Poenie, the ability to use visible wavelengths to excite the molecule and the advantages therein and the ability to produce an indicator that would resist leakage and/or bind to a cell membrane as taught by Poenie.

3. In searching this the claims of this application, examiner found the following abstract.

L23 ANSWER 16 OF 216 BIOSIS on STN
AN 2004:288730 BIOSIS
TI Near-Membrane Ca2+ Measurement with Novel Fluorochromes in Arterial Myocytes.
AU Cavalli, Maurizio [Reprint Author]; Lee, Moo Yeol; Ohkura, Masamichi; Song, Hong; Zhang, Jin; Kinsey, Stephen P; Nakai, Junichi; Kotlikoff, Michael I; Blaustein, Mordecai P
CS Physiol, U Maryland Med Sch, 655 W. Baltimore St, Baltimore, MD, 21201, USA mcava001@umaryland.edu
SO FASEB Journal, (2004) Vol. 18, No. 4-5, pp. Abst. 829.11. http://www.fasebj.org/. e-file. Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. Washington, District of Columbia, USA. April 17-21, 2004. FASEB.
AB PLasmERosomes, Ca2+ signaling complexes, consist of certain plasma membrane (PM) microdomains, the subjacent "junctional" sarco- (or endo-) plasmic reticulum, and the intervening cytosol. Ca2+ concentrations in these tiny sub-PM cytosolic spaces ((Ca2+)SPM) are apparently regulated independently of the Ca2+ in bulk cytosol. Novel "near-membrane" Ca2+ indicators should enable us to measure (Ca2+)SPM and thereby study PLasmERosome function directly. Fluo-MOMO-AM (TefLabs, Austin, TX), a fluorochrome based on Fluo-4-AM, was loaded into intact rodent small mesenteric arteries (RSMA). Confocal microscopy verified that Fluo-MOMO is anchored to PM and organelle membranes by a hydrophobic tail, and that it detects cytosolic Ca2+ signals. We also generated PM-targeted derivatives of G-CaMP, a Ca2+-sensitive dye based on green fluorescent protein (Nakai et al., Nature Biotech. 19:137, 2001). We

> fused the gene for an improved G-CaMP (G-CaMP2; with increased quantum efficiency and extinction coefficient) to the C-terminus of the gene for the Na+ pump (1 subunit that is uniformly distributed in the PM. Plasmids were transfected into intact RSMA and primary cultured artery myocytes. Confocal and wide field imaging verified the PM localization of expressed protein and its ability to detect Ca2+ signals. A gene for G-CaMP2 fused to the Na/Ca exchanger isoform 1 that is confined to PLasmERosomes was also constructed.

The date of the meeting was shortly after the filing date of the instant application. However it is clear that the authors were aware of the Fluo-MOMO-AM product that is within the scope of claim 2. Since the date of the meeting is so close to the filing date of the instant application, an issue of public use or on sale activity has been raised in this application. In order for the examiner to properly consider patentability of the claimed invention under 35 U.S.C. 102(b), additional information regarding this issue is required as follows: examiner requests information regarding the sale to and/or the disclosure of the instantly claimed product to the presenters/authors. Did it occur in a manner and time frame that would cause an on sale bar for the instant claims? If so, what was the extent of the disclosure/offer to sell?

Applicant is reminded that failure to fully reply to this requirement for information will result in a holding of abandonment.

4. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to the near membrane dyes and the structures or synthesis required.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571) 272-1265. The examiner can normally be reached on Monday-Thursday and Alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on (571) 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Arlen Soderquist
Primary Examiner
Art Unit 1797